



BioSafety Training

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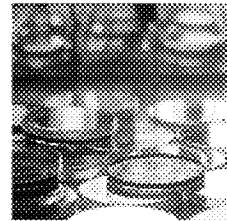
Presentation Outline

- Biosafety levels
- Human Source Material/infectious microbes
- Routes of Transmission
- Disinfection
- Biosafety Cabinets
- Accidental Spill and Exposure procedures
- Biological Waste

Biosafety Level 1



- BSL-1 Not known to consistently cause disease in normal healthy adults
 - Examples: E.coli (K-12 strains), most rDNA work, plant research labs, undergraduate teaching labs



rDNA & NIH Guidelines

- All recombinant DNA research must be registered with the Institutional Biosafety Committee (IBC)
- NIH Guidelines for rDNA research
 - Proper containment and disposal for rDNA research
 - Prevent release to environment
 - Prevent entrance to food chain
 - Report incidents (exposures/spill outside of lab) to IBC & NIH

Biosafety Level 2



- BSL-2 Moderate risk agents that cause human disease of varying severity by skin puncture, ingestion, mucus membrane exposure (splashing & infectious aerosols).
 - Examples:
 - Listeria, Salmonella, Staphylococcus aureus (MRSA, VRSA), Hepatitis A,B,C, Work with Human Source Material, HIV

Human Blood, Body Fluids, Tissues, Cell lines

Potential Pathogenic Agents

- At least 20 identified blood-borne pathogens-
Hepatitis B, Hepatitis C, HIV
- Breast Milk; Cytomegalovirus, HBV, HIV
- Fecal material; Cryptosporidium, HAV,
Salmonella, Giardia
- **Universal Precautions**
- **Hepatitis B Vaccination**

Blood-Borne Pathogens



- The Classic Blood-Borne Pathogens

- Hepatitis B
- Hepatitis C
- HIV

- Viruses

- Viral hemorrhagic fever viruses
 - Lassa, Marburg, Ebola, Crimean-Congo

- Bacteria

- Treponema pallidum
- Borrelia (tick)
- Mycobacterium leprae
- Brucella

- Parasites

- Babesia microti (tick)
- Plasmodium (mosquito) Malaria
- Trypanosoma (brucei gambiense, cruzi) (insects) Chagas Disease (zoonotic)
- Leishmania (sand fly)

- Rickettsia

- Rickettsia rickettsii (tick)

- Prions

- Creutzfeldt-Jakob

Cell Culture Risks

- Purchased cell lines may have surprises; mycoplasma, viruses
 - Epstein-Barr Virus (EBV) in breast tumors
 - ATCC does not screen for contaminating agents
- Contaminating pathogenic agents; natural often zoonotic ~20 Lab acquired infections, Lymphocytic Choriomeningitis Virus (LCMV)
- Oncogenic cell lines can cause localized tumors Ex: NIH/needle stick

Biosafety Level 2 Guidelines



- Restricted access to laboratory
- Biohazard warning signs posted
- Sharps precautions, use tools for broken glassware
- DO NOT RECAP NEEDLES
- Biosafety manual.....safety protocols for disinfection, waste, procedures
- Biosafety Cabinet for procedures likely to generate splashes or aerosols
- Training provided to personnel
- PPE provided: gloves, face shield, lab coat
- All biological waste decontaminated and stored in a leak-proof covered container

Biosafety Level 2 Guidelines



- Centrifuges must have sealed rotor heads or centrifuge safety cups
- Work surfaces are decontaminated with a broad spectrum disinfectant (10% Bleach)
- Procedures are performed to minimize splashing and aerosol generation



Biosafety Level 3



- BSL-3 Agents with a known potential for aerosol/inhalation transmission; (in addition to skin puncture, ingestion, and mucous membrane exposure) agents can cause serious or lethal infections

Example: Brucella, Mycobacterium tuberculosis, Coxiella burnetii, Francisella tularensis

Requires: negative airflow into lab, access doors self-closing and physically separated from public areas

Biosafety Level 4



- BSL-4 Dangerous or exotic agents which pose high risk of life-threatening disease, inhalation hazard; agents with unknown risk of transmission, limited treatment.
 - Examples: Ebola, Marburg Virus, Lassa Virus, Hantavirus
 - Requires a dedicated building, air-supplied suits, glove boxes

Research with Animal Models & Laboratory Animal Allergy (LAA)

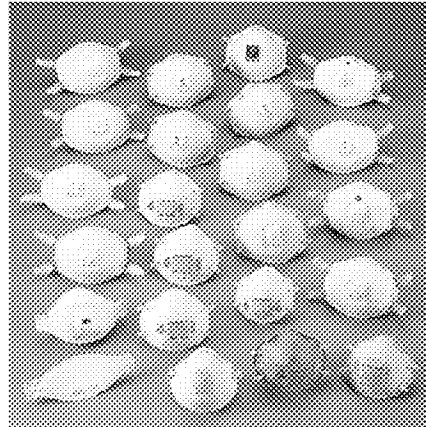
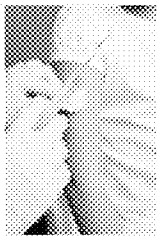
- 10-44% Animal Care Workers/researchers LAA
- 10% Develop occupational-related asthma
- 73% with pre-existing allergies=LAA
- 10% without pre-existing allergies=LAA
- Rare-anaphylaxis if bit and sensitized to saliva
- Allergy symptoms evolve over 1-2 years of exposure
- Most common allergens are found in urine
- Inhalation is the most common way allergens enter the body

Reduce exposure to animals & animal products

- Filter top or microisolator caging
- Use of personal protective equipment
 - Gloves, lab coat, scrubs, dedicated shoes or booties, goggles, respirators
- Hand washing and showering after work
- Dump stations used for discarding bedding
- Avoid wearing street clothes while working with animals
- Keep cages & animal areas clean

Occupational Health Program

- Tetanus/other vaccination
- Respiratory protection program (N-95's)
- Plan for accidental exposures
- UHS collaboration



Personal Protective Equipment

- Gloves: change frequently, never wear outside of lab, no reuse, latex/nitrile
 - Wash hands after removal
- Masks: mucous membrane protection, splashing, aerosols,
 - N-95 respirators (*requires respiratory program enrollment)
VS. Dust masks
- Eye goggles: eye protection for mucous membrane and other hazards
- Lab Coat: should not take home if working with biohazardous materials, autoclave if contaminated

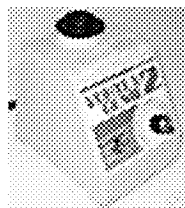
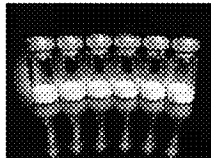
Laboratory Acquired Infections

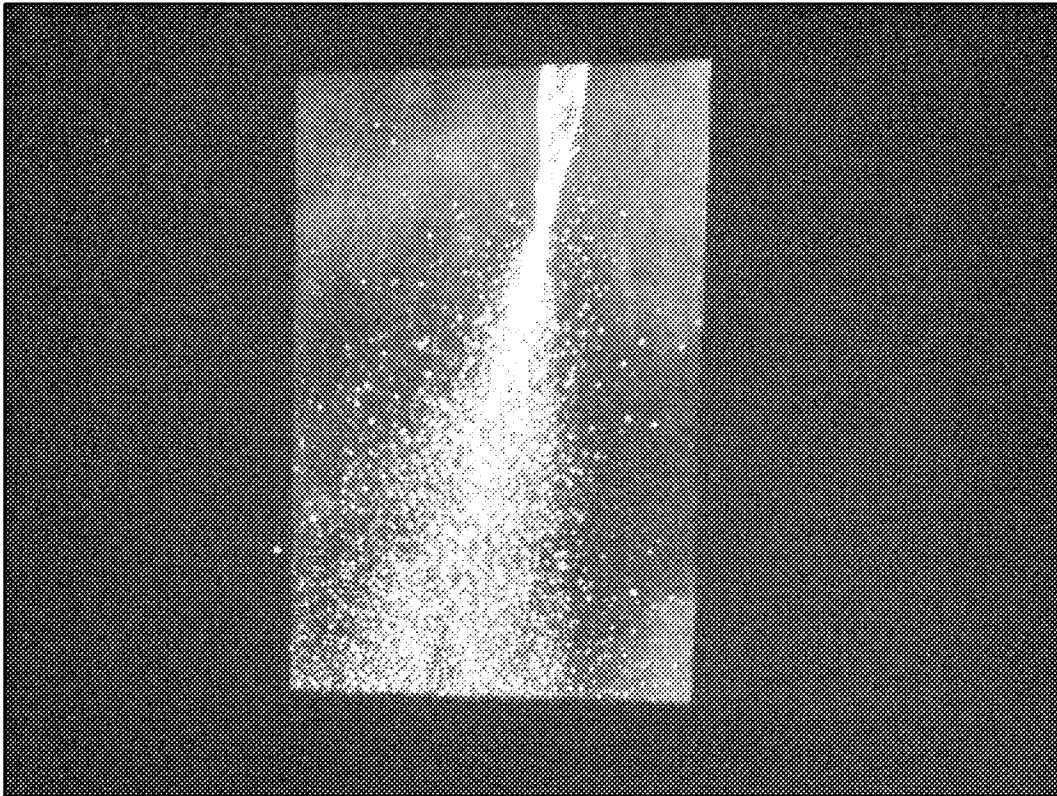
- Exposure to biological agents known to cause disease are infrequent
- The causative incident for most LAI's is unknown.....ONLY 20% are from a recognized incident.
- Less obvious exposures are probably due to inhalation of aerosols or direct contact with droplets containing infectious microorganisms

Aerosols

Produced by almost all routine techniques in the lab

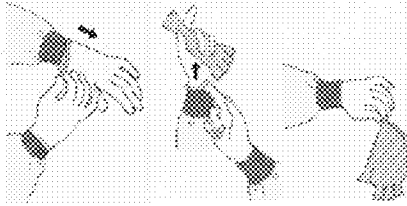
- Centrifuge
- Spraying
- Sonicator
- Vortexing
- Homogenizer
- Blender
- Fermenter
- Pipette
- Vigorous shaking
- Pouring
- Opening lyophilized cultures
- Flaming loops/needles
- Changing animal bedding





Laboratory Acquired Infection (LAI)

- Contaminated needles/sharps exposure
- Contaminated gloves
- Centrifuge Breaks
- Aerosols

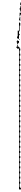


Environmental Survival

Agent	Environmental Survival
Hepatitis B	▪Survives in blood for two weeks
Francisella tularensis	▪Carcasses=133 days, water 90 days, straw=192 days ▪Shed in urine, respiratory secretions ▪5-10 organisms respiratory route for an infection
Salmonella	▪Survives for long periods of time in the environment ▪Shed in feces, urine, blood ▪Fecal/oral transmission
Lymphocytic choriomeningitis	▪Survives in mice droppings for days ▪Shed in urine, feces, saliva,

Relative Resistance of Microorganisms to Disinfection

- | | |
|---|------------------------------------|
| ▪ Prions (CJD, BSE) | ▪ Most Resistant |
| ▪ Bacterial spores (Anthrax) | |
| ▪ Mycobacterium (TB) | |
| ▪ Nonlipid viruses (Polio, Rhino viruses, Norovirus, HAV) | |
| ▪ Parasites (Cryptosporidium) | |
| ▪ Fungi (yeasts) | |
| ▪ Bacteria (E. coli) | ▪ Least Resistant to Disinfectants |
| ▪ Lipid Viruses (HIV, HBV) | |



Disinfectants

- Virkon, Wescodyne, Clidox, 10% Bleach
 - Important to read labels to determine kill claims
 - Expiration dates
 - Dwell times



Disinfectants: 10% Bleach

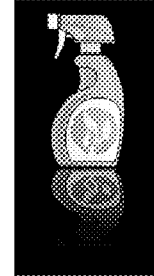
- Advantages 10% Bleach
 - A broad spectrum disinfectant
 - Kills vegetative bacteria, lipoviruses, nonlipoviruses, bacterial spores, most parasites & fungi
 - Contact time 10-30 minutes
- Disadvantages
 - A short shelf-life, must make fresh daily
 - Concentrated bleach < 2 year shelf-life
 - Corrosive (some brands use anti-corrosives)
 - Residues
 - Inactivated by organic matter
 - Skin, eye, & respiratory irritant



Limited Disinfectant: 70% Ethanol

- Advantages;

- Kills vegetative bacteria, lipoviruses
- Long shelf-life
- Noncorrosive, no residues,
- Not a skin or respiratory irritant
- Contact time 10 minutes



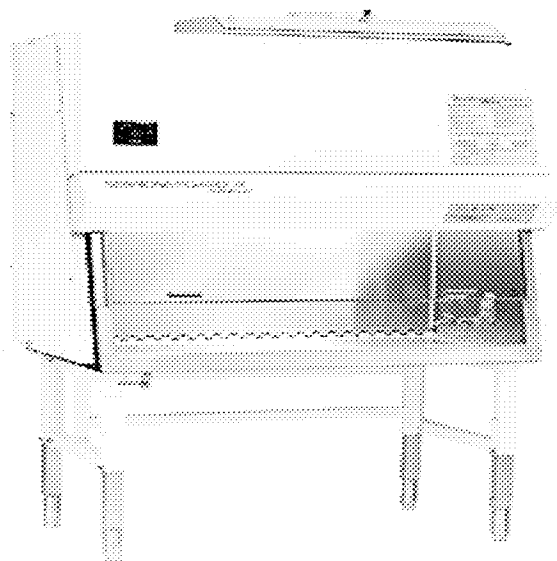
- Disadvantages;

- Not a broad spectrum disinfectant, Does not kill nonlipidviruses & bacterial spores
- Flammable
- 95% ETOH –a drying agent, not denaturing

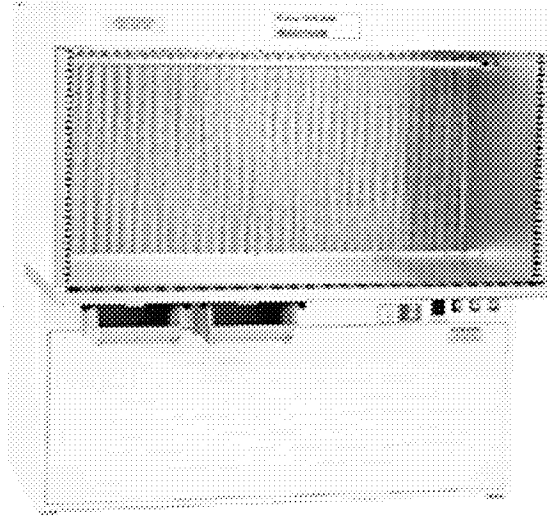
Biosafety Cabinets

- Developed for working safely with infectious materials
- HEPA filter=High Efficiency Particulate Air
99.97% min. particle removal for .3microns
- Laminar flow (Clean Benches) -for plant tissue culture, media preparation ONLY
- Fume hoods -for work with volatile chemical compounds

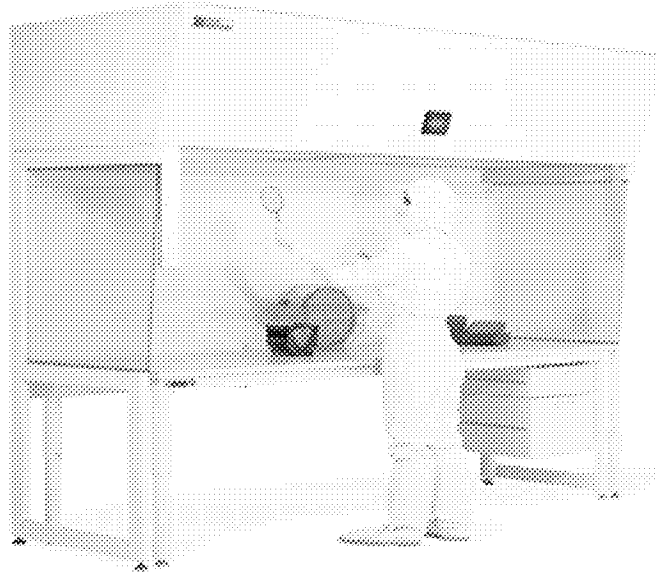
Biosafety Cabinet



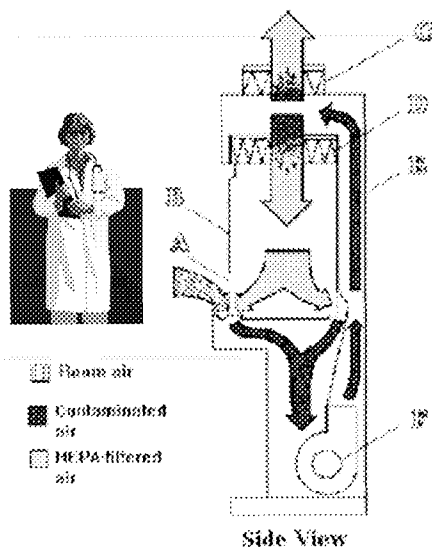
Laminar Flow or Clean Bench Horizontal flow



Laminar Flow or Clean Bench Vertical Flow

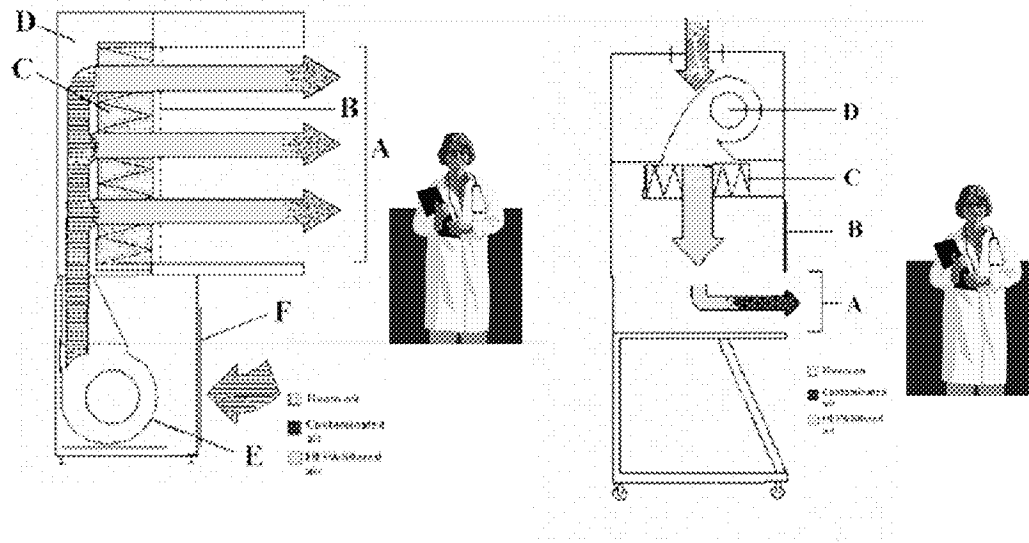


Biosafety Cabinet Airflow Diagram



Laminar Flow or Clean Benches

Airflow Diagram



Biosafety Cabinet Types

- Class I: Product protection, equipment use only, no pathogens allowed
- Class II Type A: Both personnel & product protection
 - not suitable for use of volatile and toxic chemicals
 - 30 - 70% recirculated air
- Class II Type B1: Microbes plus toxic chemical & radioactivity (None on campus yet)
 - 70% exhausted, 30% recirculated
- Class II Type B2: no recirculated air
- Class III: Totally enclosed

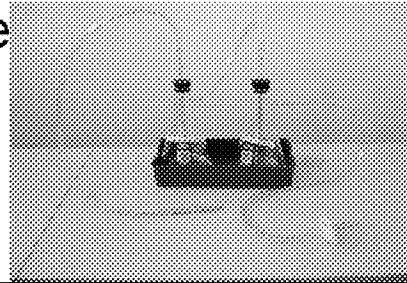
Biosafety Cabinets

Procedures for Use

- Turn on cabinet for 5 minutes before initiation
- Disinfect surfaces
- Assemble & organize material clean-contaminated areas, equipment in rear
- Wear PPE
- Slow hand & arm movements
- Do not block grilles
- Remove contaminated items after decontamination or place in sealed biohazard bags
- Do not store items in a biosafety cabinet
- Disinfect after completion & autoclave wastes

Biosafety Cabinets

- No open flames.....NO GAS
- No UV light
- Annual certification
- Re-certify if moved, or see a drop in air pressure gauge
- Protect vacuum source



Accidental Spill/Centrifuge Break

Outside of a Biosafety Cabinet with Potential Aerosols

- Dropped cage, culture, specimen, etc.
- Evacuate room (if possible) for 30 minutes to allow aerosols/dust to settle
- Place DO NOT ENTER signs on doors
- Re-Enter the facility with appropriate PPE
- Clean up using paper towels and disinfectant or a 10% bleach/10-30 minute contact time
- Reapply disinfectant to spill area, wipe up
- Place all clean-up materials in biohazard waste

Accidental Exposure

Needle, Sharps, Animal Bite/Scratch or splash to eye/mouth

- **#1:** Wash area ASAP with soap and hot water for several minutes then disinfect/ rinse eyes with saline solution

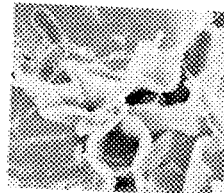
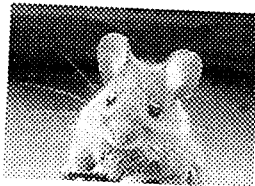
- ****5 MINUTE RULE****



- **#2:** Call Occupational Health Provider. Alert PI, Animal Supervisor, Biosafety Officer and EH&S of exposure
- **#3:** Seek medical treatment at Student Health Services or an Emergency room for post exposure evaluation/treatment within 2 hours of incident
CALL 911 if a serious accident

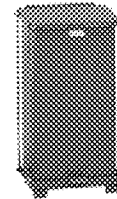
Medical Surveillance

- If immunosuppressed (taking steroids, pregnant, etc), you are more at risk
- If ill seek medical attention and inform Doctor what you work with (animals, microbial agents etc.)



Biological Waste

- All Biological waste must be kept in a leak proof and covered container labeled with a biohazard symbol.
- The container must be lined with red plastic bags with a biohazard symbol
- All biological waste must be deactivated before final disposal (autoclaved or sent off-site for incineration)



Biological Waste



- Medical Waste, Sharps & Animal Carcasses-are boxed for off-site incineration
- Sharps: Syringes, needles, razor blades, scalpel etc. Placed in a sharps container.
- NIH rDNA Guidelines require destruction of recombinant animals/plants and other agents (@UMass we autoclave)
- Stickers are placed inside the box, on the biohazard bag, with the source of the contents, i.e. Trainer Lab, 123 Daisy Hall

Ethidium Bromide Waste

- Potent mutagen/Absorbed through the skin always wear gloves, lab coat and safety goggles
- Wear UV-blocking eyewear when using UV light
- Spills clean up with soap and water
- Use a UV light to survey work surfaces
- Liquids-dispose of as hazardous waste
- Gels-collect in wide mouth jars (Dry)

Autoclave Waste

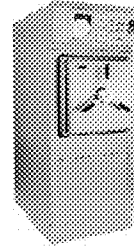


- Steam sterilization with 15 pounds of pressure per square inch @ 121C (250F), minimum cycle of 30 minutes.
- Should be autoclaved:
 - Biological material including pathogens, culture dishes & glassware, contaminated solids, rDNA, cages of BSL-2 animals
- Should NOT be autoclaved:
 - Material containing solvents, volatile or corrosive chemicals and radioactive material
- All autoclaved bags of biological waste must use autoclave tape as an indicator and be labeled with a sticker indicating autoclaved material and origin location

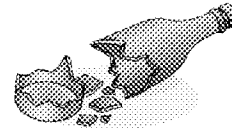
Autoclaves

Validate at least quarterly & certify annually

- Chemical/tape indicators only assure that the load reached the appropriate temperature for decontamination (not time)
- Biological Indicators: *Geobacillus stearothermophilus* (Prospores), indicate that both temperature and time have been reached for decontamination



Autoclaves



- Maintain autoclave logs:
 - Time, temperature, validation & maintenance records, load description, name/signature
 - Biological indicator verification monthly
 - Annual autoclave certification by a licensed professional

For proper steam penetration:

- Do not pack contents too tightly
- Tie bags loosely
- Loosen Caps! Wear PPE when opening door!

Contact Information

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